University of São Paulo

School of Pharmaceutical Sciences of Ribeirão Preto

Graduate Program in Biosciences and Biotechnology

Abstract collection

II WORKSHOP OF BIOSCIENCES AND BIOTECHNOLOGY

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Scientific Program

Thursday - 06/12/2018

13:40H - WELCOME SESSION

14:00H-15:00H - LECTURE 1: "HOW CARCINOMAS BECOME AGGRESSIVE", PROF. DR. ROBERT WEINBERG, DANIEL K. LUDWIG PROFESSOR FOR CANCER RESEARCH; MEMBER, WHITEHEAD INSTITUTE, MIT, MASSACHUSETTS, USA.

15:00H-16:00H - LECTURE 2:"AUTOPHAGY IN DIFFERENTIATION AND HOMEOSTASIS OF IMMUNE CELLS", PROFA. DRA. ANNA KATHARINA SIMON, KENNEDY INSTITUTE OF RHEUMATOLOGY, UNIVERSITY OF OXFORD, LONDON.

Friday - 07/12/2018:

9:00H-12:00H - POSTER SESSION

14:00H-15:00H - LECTURE 3: "VACCINE DISCOVERY ON A VIRUS-LIKE PARTICLE PLATFORM", PROF. DR. DAVID PEABODY, MOLECULAR GENETICS AND MICROBIOLOGY, UNIVERSITY OF NEW MÉXICO, USA.

15:00H-16:00H - ORAL PRESENTATIONS (SELECTED PHD STUDENTS)

16:30H-17:00H - CLOSING SESSION.

Abstracts

P01 - Expression of recombinant protein from Dengue, Zika and Chikungunya viruses to differential serological diagnosis

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The arboviruses dengue, zika and chikungunya represent a serious public health problem in Brazil and in the world. They circulate simultaneously due to the common vector, Aedes mosquito, and are responsible for thousands of annual reports of infections in Brazil with serious consequences as death of many individuals. These diseases have similar symptoms which impair the exact clinical diagnosis. The specific diagnosis is necessary to avoid aggravating the consequences that each one can bring to the patient. Serological diagnostic tests available on the market identify a single virus at time. Discriminatory tests are molecular assays like real time PCR. Such methods are excellent but inadequate for mass diagnosis due to cost. Because of the risks of death or sequels that these viruses impose on individuals, a discriminatory diagnosis is necessary, and the improvement of tests is desired.

The genes of the proteins ZIKVNS1, DENVNS1, DENVE3 and CHIKVE2 were successfully cloned in different strains of Escherichia coli, but the CHIKVE2 protein was not expressed in any of the strains. ZIKVNS1, DENVNS1 and DENVE3 proteins were produced in Rosetta (DE3) bacteria transformed with pET28a recombinant vectors. These three proteins were solubilized from inclusion bodies, purified by nickel affinity chromatography and identified by SDS-PAGE and Western Blotting. The DENVNS1 protein was recognized in a rapid test for detection of dengue NS1 antigens, which gives it specificity and great diagnostic potential as capture antigen. In this work the recombinant proteins DENVNS1, ZIKVNS1 and DENVE3 were successfully expressed in E. coli system and this is the first step in the production of a fast, discriminatory, unified diagnostic device produced with national technology, reducing the costs of importing these products. These rapid tests can broad the diagnostic cover.

Keywords: Recombinant protein; Arboviruses; Diagnostic; Escherichia coli.

Financial Support: CAPES

P02 - Salmonella Typhimurium strains isolated from humans showed higher virulence than strains isolated from food in Brazil in the Galleria mellonella infection model

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Salmonella enterica subsp. enterica serovar Typhimurium (S. Typhimurium) is one of the main foodborne enteropathogens in various countries, including Brazil. The virulence of this pathogen has been studied in mice, but there are few studies using an alternative infection model such as Galleria mellonella. The aim of this study was to analyze the virulence of S. Typhimurium strains in G. mellonella infection model. A total of 13 S. Typhimurium strains isolated from humans (7) and food (6) between 1983 and 2013 in Brazil were studied. Groups of 10 larvae were infected with each strain of S. Typhimurium. For artificial inoculation of each larva, 5 µl (solution 100 CFU) were injected across center of the last right pro-leg with a Hamilton microliter syringe. After the inoculation, the larvae were incubated at 37°C, deprived of feed and direct illumination. The same was done for the negative control using PBS and for the positive control infected by S. Typhimurium ATCC 14028 strain. The larvae survival was scored daily for 7 days. Statistical analyses and graphics were performed by the Log-rank (Mantel-Cox) method in Prism 5 software (GraphPad®). The ATCC 14028 strain and three strains, being two isolated from humans and one from food were highly virulent killing 100% of larvae during 7 days. Other four strains from humans showed an intermediary pattern killing 70 – 90% of the larvae in 7 days. However, four strains isolated from food and one from human were less virulent killing 10 – 20% until the end of the experiment. Finally, one strain isolated from food was avirulent. In conclusion, G. mellonella larvae showed to be a good alternative model of infection to study S. Typhimurium strains during various days revealing that S. Typhimurium strains isolated from humans showed a higher virulence than strains isolated from food in Brazil.

Keywords: Salmonella Typhimurium; Galleria mellonella; virulence; humans; food

Financial support: CAPES and FAPESP

P03 - THE INFLUENCE OF OBESITY IN IMMUNO RESPONSE IN RATS INFECTED BY TRYPANOSOMA CRUZI

Amanda Goulart[1], Pedro Alexandre Sampaio Oliveira[1], Vânia Brazão[1], José Clóvis do Prado Júnior[1].

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Chagas disease (CD) is a neglected tropical disease caused by Trypanosoma cruzi. CD is still considered neglected and causes about 10,000 deaths per year. In Brazil there are between 2 and 3 million people with CD. Annually, more than 12,000 deaths occur worldwide. The mechanisms that contribute to the pathological processes observed in the organs affected during the T. cruzi infection are not yet completely elucidated, however, it is known that there is a complex interaction involving cellular lesions, inflammatory response and fibrosis. Obesity is a global problem and affects about 107.7 million children and 603.7 million adults, predisposing people to diseases such as cancer, type 2 diabetes, liver disease, osteoarthritis, and cardiovascular disease. Studies indicate that during chronic T. cruzi infection, adipocytes act as reservoirs of the parasite for long periods of time. After initial infection, adipose tissue presents a generalized invasion of macrophages and a reduction in lipid accumulation and adipocyte size, a process that results from the increase of oxidative stress and the expression of lipolytic enzymes. Thus, adipose tissue is also an important escape mechanism for the parasite of the immune response. Therefore, more studies are needed for the evaluation of inflammatory mechanisms in adipose tissue during CD. The Aim of this work is to evaluate the influence of obesity on the immune response in T. cruzi-infected rats. Male Wistar Hannover rats will be used and will receive water and feed ad libitum. Obese groups will receive the feed supplemented with a cafeteria diet. The animals will be intraperitoneally (i.p.) infected with a dose of 1 x 105 trypomastigote forms of T. cruzi Y strain present in whole blood. The interest assays will be performed after 12-14 days of the inoculum and the plasma and tissue samples will be collected for analysis.

Keywords: Chagas Disease; Obesity; Immune Response

Financial Support: CAPES, FAPESP, CNPq

P04 - Development of real-time RT-PCR for differential diagnosis of circulating arboviruses in Brazil

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Aims: Arthropod-transmitted viruses are classified informally as arboviruses. More than 150 arboviruses infect humans causing febrile illness with general malaise, as well as hemorrhagic and central nervous system diseases, becoming fatal in some cases. Arboviroses are of great importance for public health in countries like Brazil. The most widespread viruses in Brazil today are: dengue (DENV), Chikungunya (CHIKV), Zika (ZIKV), Oropouche (OROV) and Yellow Fever (YFV). Others of clinical relevance have already been isolated in Brazil, such as Saint Louis encephalitis virus (SLEV), Rocio (ROCV), Cacipacore (CPCV), Ilhéus (ILHV) and Mayaro (MAYV). The development of highly sensitive and specific methods to detect and confirm the infection caused by these viruses is of paramount importance for early diagnosis, allowing better clinical follow-up of the infected and preventive measures to reduce the risks of epidemics. The objective of this work is to develop real-time RT-PCR methods in a single step using specific primers and probe for CPCV, ILHV, MAYV, YFV, SLEV, OROV and WNV.

Methods: Primers and probe for each of these viruses were designed by selecting conserved regions from the alignment of viral genomic sequences deposited in GenBank using the CLC bio software. The primers were designed for amplification of genomic regions of 120-200 base pairs, and the probes in regions internal to these.

Results: The primers and probe were used to standardize a real time RT-PCR for the specific detection of YFV, using RNA purified from the supernatant of C6/36 cells infected with YFV. An agarose gel electrophoresis showed an amplification product of 170 base pairs compatible with the expected size for YFV. The real time RT-PCR did not show cross reaction with DENV1, DENV2, DENV3, DENV4 and ZIKV, the main flaviviruses circulating in Brasil.

P05 - Diversity of high-level aminoglycoside resistance mechanisms among Gram-negative nosocomial pathogens in Brazil

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Introduction: High level aminoglycoside resistance in Gram-negative bacilli may also be conferred by production of multiple AMEs or increased efflux. Studies on HLAR, especially pertaining to 16S-RMTases, have mostly involved the Enterobacteriaceae, whereas comparable data on lactose-non-fermenting Gram-negative bacilli remain scarce. Objective: The aim of this study was to elucidate the mechanisms of HLAR among Gram-negative nosocomial pathogens in Brazil. Gram-negative clinical bacterial isolates (n=107) from cerebrospinal fluid, blood, urine, resistant to oxyimino-cephalosporins and/or aztreonam screened from three States in Brazil from 2007-2014 were investigated. Methods: Disk diffusion tests and the minimal inhibitory concentrations by broth microdilution were performed. Efflux inhibition assay was carried out using the efflux pump inhibitor phenylalanine-arginine β-naphthylamide (PAβN) for isolates without 16S-RMTase gene. MIC testing (amikacin/gentamicin) was performed in the presence and absence of 50µg/mL PAβN, and a minimal 4-fold reduction in the MIC values in the presence of PAβN was considered significant. PCR and sequencing for detection of 16S-RMTase genes was performed. The isolates with negative PCR results were subjected to whole genome sequencing (WGS). Results: Nineteen of the 107 strains were highly resistant to gentamicin, tobramycin and amikacin with MICs of >128 µg/ml, consisting of 14 Pseudomonas aeruginosa, 3 Acinetobacter baumannii and 2 Klebsiella pneumoniae. Ten of the 19 strains carried the 16S-RMTase genes (rmtD, 8 and rmtG, 2 isolates). Upon whole genome sequencing, the remaining 9 isolates (6 P. aeruginosa and 3 A. baumannii) did not contain any known 16S-RMTase genes or their homologues, but instead carried combinations of 13 AME genes (aacA4, aacA1, aacC1, aphA7, aphA6, aph(3')-IIb, aadB, aadA1, aadA2, aadA6,

aadA7, strA and strB) that collectively accounted for the high-level aminoglycoside-resistant phenotype. Aminoglycoside resistance was inhibited by PAβN in only one A. baumannii isolate. Both arbekacin MIC >256µg/ml and the absence of inhibition zone were highly sensitive and specific in predicting the presence of an 16S-RMTase gene. Conclusions: HLAR among GNB in Brazil is due to production of 16S-RMTase or combination of multiple AMEs, while involvement of efflux appears to be minimal. Combination of AMEs was particularly common among P. aeruginosa and A. baumannii leading to HLAR phenotype. High-level resistance to arbekacin could be used as a marker to differentiate these two resistance mechanisms among these species.

Keywords: HLAR; AMEs; 16S-RMTase; gram-negative bacilli; arbekacin

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P06 - Evaluation of the role of galectin-1 and -4 on experimental infection by Leishmania (Leishmania) amazonensis

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Leishmaniasis is a neglected disease caused by protozoa of the genus Leishmania. This infectious disease is a worldly serious health problem with ineffective treatment and increasing incidence. Studies have shown that the resolution of this infection is dependent of homeostatic immune response from the host. However, there are still many gaps in understanding its pathogenesis. The role of galectins, proteins that recognize glycans with beta-galactoside motifs, have been described in several infectious diseases. Nevertheless, there are few reports on the involvement of galectins in Leishmaniasis. Therefore, the aim of this work is to evaluate the role of galectins 1 and 4 in experimental infection by Leishmania sp. We demonstrated that galectin-1 (Gal-1) deficiency in mice with the BALB/c background (Lgals1-/--BALB/c), whether not in Lgals1-/--C57BL/6, promotes restriction of L. (L.) amazonensis infection. This outcome was related to increased IFN-γ production and decreased IL-4, IL-10, IL-12p70, and TNF-α and decreased lesion size and parasite load at the draining lymph nodes. Macrophages deficient in Gal-1 from BALB/c background promotes control of parasite replication inside host cells compared with wild type macrophages. Gal-1 did not bind to this parasite, suggesting that the susceptibility of wild type BALB/c was not due to a direct interaction between galectin-1 and Leishmania. Full length Gal-4, and not their truncated forms, recognizes L. (L.) amazonensis in dose and carbohydrate-dependent manners. Furthermore, Gal-4 recognizes lipophosphoglycan (LPG) at the surface of L. (L.) amazonensis. This interaction is not essential for parasite internalization but is important to killing the parasite inside macrophage. More studies on the involvement of Gal-4 in murine leishmaniasis will be developed. These results demonstrate that galectins can participate in the immune response against L. (L.) amazonensis and open new approach for therapeutic or diagnostic strategies applicable to this neglected disease.

Keywords: Leishmania; Leishmaniasis; Galectins; Lipophosphoglycan; immune response.

Financial Support: CNPq and CAPES.

P07 - Recombinant human granulocyte colony stimulating factor (rhG-CSF) expression in HEK293 cells, characterization and a comparison with the rhG-CSF produced in Pichia pastoris system.

Bruna Samhan Archangelo; Elisa Maria de Sousa Russo; Virginia Picanço Castro; Kamilla Swiech Antonietto

Human granulocyte colony stimulating factor (hG-CSF) acts primarily to promote the maturation of neutrophils (granulocytes) and stimulate their phagocytic and chemotactic activity. hG-CSF has been used in the treatment of various pathologies, especially neutropenia caused by events that suppress the production of myeloid cells. The country would benefit greatly from the national production of this drug, since a large part of the acquisition costs are attributed to imports. The recombinant protein production demand in mammalian cells has increased, mainly due to the increase in the number of approvals molecules as biopharmaceuticals. Increasing interest in this kind of technology has resulted in improved production efficiency as well as the quality of products obtained from their expression systems. The great advantage of using these cells as an expression system is that they are capable of performing post-translational modifications and correct protein folding, generating a protein with very similar characteristics to those present in the human organism. Our group has already performed the cloning and expression of rhG-CSF in P. pastoris. The present project aims to produce rhG-CSF in HEK293 cells in serum-free medium, perform in vitro and in vivo biological function tests, and perform an analysis of the glycosylation patterns of both the recombinant protein produced in HEK293 cells and that produced in P. pastoris system. The objective is to obtain the potential of application of each expression system, with evaluation of the production yield and preservation of the therapeutic characteristics of rhG-CSF. This work therefore aims to improve methods for the development and production of rhG-CSF protein nationally. In addition, it aims to generate knowledge to assist in the consolidation of the use of human cells for the production of therapeutic proteins. Three different strategies were adopted for the construction of the clones to be used in this project. The first was the cloning of the hG-CSF gene into the 1054 lentiviral expression vector. The other strategy was the cloning of the gene into the plasmid expression vector pcDNA3.1 and, finally, the cloning of the rhG-CSF synthetic gene codon optimized for expression in human cells. The expression plasmids were constructed and, currently, the production of the viral particles by the lentiviral approach is being performed.

Keywords: Biotechnology; recombinant protein; biopharmaceutical; bioprocess; recombinant human granulocyte colony stimulating factor, HEK293

Financial Support CAPES

P08 - Evaluation of the effect of hormonal contraceptives on the interaction among neutrophils, the complement system and the vascular endothelium

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Introduction: Venous thromboembolism is an important global health problem. Neutrophil plays a fundamental role in the innate immune system, among their functions, we highlight the neutrophil extracellular traps (NETs), which may also promote inflammatory and thrombotic disorders. Our research group has studied the effect of combined hormonal contraceptives (CHC) on complement system (CS) and hemostasis. There is a very close physiological relationship between CS and hemostasis, sharing a complex network of interaction/regulation between their components. However, there are no studies relating the effect of CHC hormonal components on the interactions among CS, neutrophils and endothelium, which may contribute to the understanding of the action of female hormones on the pathophysiology of thrombosis by using CHC. Aim: To evaluate the effect of CHC components on the interaction among neutrophils, components of the complement system and vascular endothelium. Methods: The model proposes the exposure of endothelial cells to neutrophils stimulated by antigen-antibody complexes in the presence of the CS and the most common hormonal components in the 2nd and 4th generation CHC. The effect of the hormonal components will be evaluated by the measurement of neutrophil oxidative metabolism activation, degranulation, NET release, lipid peroxidation of endothelial cells, activation of endothelial cells measured by the release of soluble adhesion molecules and activation of the complement system.

Keywords: neutrophils, complement system, endothelium, thromboembolism, contraceptives.

Financial Support: CAPES

P09 - HIGH SURVIVAL RATES OF CAMPYLOBACTER COLI UNDER DIFFERENT STRESS CONDITIONS HIGHLIGHT THE PATHOGENIC POTENTIAL OF BRAZILIAN STRAINS

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Campylobacter spp. is an important causative agent of human diarrheal diseases worldwide. AIMS: In this study it was assessed the frequency of some virulence genes and survival under different stress conditions of C. coli isolated from different sources in Brazil. METHODS: A total of 50 C. coli strains isolated from humans (12), animals (15), the environment (15) and chicken meat (8) between 1995 - 2011 in Brazil were analyzed for their frequency of 16 virulence genes, tolerance to temperature variations (4oC and 37oC), survival in the presence of 7.5% of NaCl and under acid and oxidative stress conditions. RESULTS: All strains studied presented the cadF, flaA, and sodB genes. The cdtB gene was detected in 13 (26%) strains, flhA gene in 12 (24%), dnaJ gene in ten (20%), pldA gene in eight (16%), iamA gene in three (6%); genes docA and cdtC were detected in two (4%) strains and the gene cdtA was found in only one (2%). The genes racR, virB11, csrA, wlaN and ciaB were not detected. All strains grew at 4°C and 37°C after 24 hours. All strains studied remained 100% viable after the first hour of incubation in BHI with a final concentration of 7.5% NaCl and in acid BHI (pH=4.5). Sixteen of the 23 strains submitted to oxidative stress survived after 10 minutes of incubation. CONCLUSIONS: In conclusion, the pathogenic potential of the strains studied was reinforced by the presence of some important virulence genes and by the high growth and survival rates of the majority of those strains under different stress conditions. Once temperature variations, NaCl, changes in pH and oxidative stress are methods widely used to control bacterial growth, the results obtained might suggest that more rigorous control measures may be needed given the importance of contaminated food as vehicles of Campylobacter coli.

Keywords: Campylobacter coli, Pathogenic Potential, Different Stress Conditions

Financial support: FAPESP (Sao Paulo Research Foundation)

P10 - Determination of the epigenetic signature related to human neutrophil polarization to N1 and N2 profiles

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Aim. Cellular plasticity of innate and adaptive immune cells may be under epigenetic control, so our aim is to evaluate which epigenetic changes are related to neutrophil polarization and define the epigenetic signature of neutrophils generated in the presence of polarizing cytokines of the N1 and N2 profiles. Methods. Peripheral blood from healthy donors will be collected (10 mL) for cell separation, in which the polymorphonuclear enriched portion will be isolated through the Percoll density gradient. Cells obtained will be evaluated by flow cytometer to confirm the enrichment and cell purity. For polarization, the isolated cells will be incubated with conditioned medium N1 (GM-CSF and IFN-γ) and N2 (IL-4, IL-13 and TGF-β) by at least 30 minutes, accordingly to the experiment. For the definition of the phenotypic signature, cells will be analyzed under optical microscopy for morphology, the supernatant will be collected for analysis of cytokines by ELISA and qPCR and reactive oxygen species (ROS) will be analyzed by chemiluminescence monitored through the luminometer. To epigenetic signature determination, RNA from polarized neutrophils will be extracted for the analyses of epigenetic enzymes expression by qPCR and the DNA will be analyzed for the global and specific methylation profile. Histone methylation and acetylation will be evaluated by chromatin immunoprecipitation of specific genes regions.

Keywords: epigenetic; innate immunity; neutrophils; cell plasticity

Financial Support: CAPES; CNPq; FAPESP

P11 - Metataxonomics analysis indicates a core bacterial microbiome for selected Brazilian cheeses and dairy processing environment

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There is an increasing interest in elucidating the prevalence of pathogens and unravelling the accompanying microbiota of dairy products, to improve their safety and quality assessment. Although Brazil ranks among the main global fluid milk and cheese producers, the molecular profile of autochthonous microbial communities of local dairies has not been determined. In this research, selected food and environmental samples from two Brazilian dairies were analyzed for bacterial diversity by high-throughput DNA sequencing (HTS) based on 16S rRNA genes. The samples were allocated in four groups: raw material, final product, food contact surfaces and non-food contact surfaces. Final product and non-food contact surface contained the richest and most diverse bacterial groups, as measured by alpha-diversity indexes. With regard to dissimilarity, beta diversity was low and there were two main clusters, one formed by raw material-food contact surface and the other comprising final product and non-food contact surface. The bacterial community was dominated, in a crescent order, by Macrococcus, Alkaliphilus, Vagococcus, Lactobacillus, Marinilactibacillus, Streptococcus, Lysinibacillus, Staphylococcus, Clostridium, Halomonas, Lactococcus, Enterococcus, Bacillus and Psychrobacter. These results highlight the importance of the processing environment for the cheese microbiota and indicate that with HTS it is possible to detected rare taxa of low or difficult detection by classical methods.

P12 - Antiapoptotic Effect of Ghrelin in Chronic Phase of Chagas Disease

Lessa, D.F.S.; Silva, F.P.; Bronzon da Costa, C.M.; Pereira, L.M.; Prado Júnior, J.C.; Carraro Abrahão, A.A.

Chagas disease represents a serious health problem in Latin America. Ghrelin is a peptide hormone involved in various physiological functions with anti-inflammatory, anti-oxidant and anti-apoptotic action. The objective of this work was to evaluate the effects of ghrelin on the stages of splenic apoptosis in the chronic chagasic infection of male Wistar rats. Twenty male Wistar rats with initial weight of 100g were divided into 4 experimental groups: control (C), control treated with ghrelin (CG), infected (I) and infected treated with ghrelin (IG). The animals were infected with the Y strain of Trypanosoma cruzi (2x105 trypomastigote forms); the treatment with ghrelin was started 150 days after infection for 3 weeks (100 µg / kg / day, every 2 days), subcutaneously. After treatment the animals were killed by decaptation with prior anesthesia (isoflurane) and the spleens were collected. Characterization of apoptosis stages in splenic cells and cell culture for 24 hours (without and with T. cruzi stimulation) was assessed by annexin-V (FITC) and propidium iodide (PI) labeling and analyzed in a flow cytometer (BD FACS-Canto, FACSDiva software). The percentage of annexin V+PI- cells (recent apoptosis stage), annexin V+PI+ (late stage of apoptosis) and annexin V-PI- (viable cells) were identified. The animals infected and treated with ghrelin had a reduction in the percentage of cells in initial and late apoptosis (basal and cell culture) when compared to infected and untreated animals. IG group had a high percentage of viable cells. Thus, our results showed the anti-apoptotic action of ghrelin in chronic experimental Chagas' disease.

Key words: ghrelin; apoptosis; Chagas disease; rat.

Financial Support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP - Proc. 2014 /18682-3) and CAPES.

P13 - Immune dysfunction and immunomodulatory effects of hypomethylating agents on monocytes from MDS patients

Fabiana Albani Zambuzi[1], Ricardo Cardoso Castro[1], Juan Luiz Coelho da Silva[2], Maira da Costa Cacemiro[1], Caroline Fontanari[1], Fabíola Traina[2], Flávio da Silva Emery[1], Fabíola Attié de Castro[1], Fabiani Gai Frantz[1]

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Aim: Based on evidences that immunological pathways contributed in Myelodysplastic syndromes (MDS) pathogenesis and prognosis and that hypomethylating agents have immunomodulatory effects, by affecting immune cellular components, we aimed to evaluate immunological markers in MDS patients and to determine the effect of the treatment with hypomethylating agents on monocytes functions.Methods: For this purpose, 14 MDS patients were recruited in Hematology outpatient of HCFMRP and 20 healthy individuals atUSP community. Peripheral blood was collected for plasma separation and cytokines quantification. From blood, monocytes were isolated, treated in vitro with Decitabine and theninfected with Mycobacterium tuberculosis (Mtb). After infection, cell functions were evaluated through phagocytosis and microbicidal assay and cytokine production. Results: MDS patients were diagnosed according to WHO 2017 criteria in the following entities: 5 with MDS with single lineage dysplasia, 5 with MDS with multilineage dysplasia, 3 as MDS with excess blasts and one with MDS with ring sideroblasts. Initially, we quantified plasma cytokines to identify immune profile of these patients and observed a reduction in the levels ofIFN-γ, TNF-α, IL-8, IP-10, IL-1β, CCL5, IL-4, IL-17, IL-2 and IL-12 when compared to control, suggesting an immune dysfunction of MDS patients. Also, cytokines presented a positive correlation with platelet count. When we evaluate the effect of hypomethylating agents on monocytes infected in vitro with Mtb, from healthy and MDS group, the treatment with Decitabine 5μM modulate phagocytic and microbicidal capacity only in healthy individuals. Also, the cytokine produced by infected monocytes from patients was not modulated. Conclusion: Together, our data indicated that MDS patients presented an immune dysfunction related to monocytes function that was not reversed or modulated in vitro by the treatment with epigenetic modulator drug.

Key words:Cytokine, decitabine, myeloid malignancies

Financial support: CAPES, CNPq and FAPESP (Grantnumber 2017/05365-8)

P14 - Construction of an arbovirus (Dengue and Zika) detection system in Aedes aegypti by immunochromatography

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Arboviruses are viruses that need arthropods to infect a vertebrate host. Currently, they constitute a large part of the emerging viral diseases, and therefore have a great epidemiological impact. Dengue virus, among the emerging arboviruses in Brazil, has a potential for lethality due to a unique clinical condition of the disease called hemorrhagic fever. Zika virus has lower lethality, but its also clinically important, once it has been related to Guillain-Barré syndrome and microcephaly in neonates from infected mothers. Both Dengue and Zika viruses have Aedes aegypti as a common host, which makes it an alternative control for these diseases, since there are no safe vaccines or specific treatments available. In order to control the vector of these diseases, Forrest Innovations Ltd®, in collaboration with SEBRAE, is developing sterile modified male mosquitoes to be released in high proliferation sites in order to reduce the virus circulation levels. The effectiveness of this approach will be assessed by an immunochromatography device to detect Dengue and Zika viruses in Aedes aegypti mosquitoes constructed by our research group. Dengue and Zika virus peptides will be designed and synthesized, in order to select monoclonal antibodies that recognize both viruses. The prediction of immunogenicity will be performed in silico by the Bepipred method and the synthesis of the peptides will be performed by Boc / Bzl method. The production of monoclonal antibodies will be carried out according to standard procedures and characterized by isotype through the use of a commercial kit. Their specificities will be tested against different arboviruses through ELISA. For the assembly of the immunodetection apparatus, the monoclonal antibodies will be inserted in the commercial immunochromatography kit. This work may open new approaches applicable to combat these arbovirus transmissions through the control of the mosquito infection.

Keywords: Dengue; Zika; arboviruses; Aedes aegypti; antibodies; immunochromatography.

Financial support: CAPES

P15 - Prospecting the transcriptome from Mycobacterium tuberculosis infected mice for the validation of epigenetic markers related to protection in tuberculosis

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Aim. In experimental TB the liver appears to present a very efficient response against infection when compared to the lung and spleen, presenting colony forming units counts of 1,000 to 10,000 times lower than in the lung. Our hypothesis is that in the liver, certain signalling pathways or effector mechanisms are quite efficient for the control of infections and could not be activated in the organs where the bacillus escapes from the immune system. Methods. We used RNA-sequencing (RNA-seq) analysis to identify possible factors in host organs determining the differential pathological responses in an in vivo model of mycobacterial infection. Through bioinformatics software analysis we have identified differentially expressed genes (DEG), which possibly characterize the different responses between the organs against Mtb infection. Thus, once detected in silico these enzymes associated with protection or susceptibility, the targets will be validated in vitro. PBMC cells of healthy donors will be infected in vitro with M. tuberculosis and will be treated with inhibitors of the enzymes. After inhibition of these enzymes, the immune response and bacterial growth will be evaluated. Results. We observed that important pathways and mechanisms that were activated in the liver, were not activated in the lung, such as RUNX1 that regulates transcription of genes involved in interleukin signaling, NOTCH1 Intracellular Domain, and SUMOylation of intracellular receptors. As reported in the literature, the gene expression can be modulated by epigenetic mechanisms, and accordingly different epigenetic modulation was also observed among the organs resulting in the activation of enzymatic mechanisms such as HDACs deacetylate histones.

Keywords: Tuberculosis, Epigenetic, Liver, Epigenetic enzymes, Transcriptome, M. tuberculosis.

Financial Support: Capes; FAPESP (2018/14968-0 e 2017/05365-8)

P16 - In vitro and in vivo trypanocidal activity of phenothiazine dyes against Trypanosoma cruzi

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Benznidazole and Nifurtimox are still the only antitrypanosomal medicines available to treat Trypanosoma cruzi infection more than 100 years after the Chagas' disease discovery. Both drugs display toxicity and low activity during the chronic phase of the disease. In this context, several classes of compounds have been studied to prospect molecules potentially active against T. cruzi. Phenothiazine dyes, especially methylene blue, represent a group of compounds very important for the treatment of malaria and have been described by activity against many pathogenic microorganisms and trypomastigotes forms of T. cruzi. Therefore, the aim of this study was to evaluate in vitro and in vivo trypanocidal activity from four commercial compounds phenothiazines (methylene blue-MB, new methylene blue-NMB, toluidine blue-TBO and 1,9-dimethyl methylene blue-DMMB). Cytotoxic activity of these dyes was determined in LLC-MK2 mammalian cells and the biological assays evaluated against amastigote forms of T. cruzi (Tulahuen strain) from colorimetric methods in vitro. Drug combinations were also performed to determine possible synergistic effects. Two of the most active compounds in vitro were evaluated in mice experimentally infected with strain Y. All compounds showed low cytotoxicity and higher trypanocidal activity than Benznidazole, especially MB and DMMB. There was synergistic activity among phenothiazines, but this effect was not observed when dyes were associated with benzonidazole. Although MB and DMMB were the most active compounds in vitro, they did not reduce the parasitemia of mice in the acute phase of infection, even when associated each other. Methylene blue is already a drug used in humans and has a low cost and toxicity. Therefore, other treatment conditions will be performed to understand the low in vivo activity as opposed to in vitro, because the results may contribute to development of alternative therapies for the treatment of Chagas' disease.

Keywords: Chagas' disease, Treatment, Phenothiazine dyes, drugs screening, in vivo assays

P17 - Biosynthesized silver nanoparticles for using in photodynamic therapy with methylene blue against pathogenic fungi

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The emergence of new species of pathogenic and resistant fungi to commercial drugs leads to the need to develop new strategies for treating fungal infections. The antimicrobial photodynamic therapy (APDT) is a promising technique to control resistant microorganism. The conjugation of photosensitizer (PS) (e.g. methylene blue – MB) with a nanoparticle is a process that aims to optimize the efficiency of the process. In this study silver nanoparticles (AgNPs) were biosynthesized by Fusarium oxysporum. The AgNPs were characterized by using Spectrophotometry Uv-Vis to measure surface plasmon peak, and dynamic light scattering method to obtain diameter and zeta potential. The microdilution broth method was used to determine the minimal inhibitory concentration (MIC) of MB and AgNPs in APDT. The conjugation of AgNPs and MB was performed with final concentrations of 100 µg/mL, and 50, 100, 200, 400 and 600 µM, respectively. AgNPs presented a surface plasmon peak around 420 nm, hydrodynamic diameter of 86,72 nm and zeta potential of -28,6 mV. The MIC of MB in APDT at 15 J cm-2 and AgNPs against Fusarium keratoplasticum and Candida albicans were 20,5 µM and 8,2 µM, and 5 µg/mL and 2 µg/mL, respectively. The UV-vis absorption spectra of the conjugates have shown that in 50 and 100 µM of MB, AgNPs was notable at 420 nm, while the presence of MB (655 nm) has been shown with 100 µM of MB. With 200 and 400 µM of MB, AgNPs and MB appeared at 420 and 655 nm, respectively. The absorbance of free MB is next to absorbance of MB in the conjugate. 600 µM of MB showed no evidence of AgNPs and absorbance of MB conjugated is lower than free MB. Thus, AgNPs have antifungal effects and present a high potential to optimize photodynamic therapy.

Key words: silver nanoparticles; biosynthesis; antimicrobial photodynamic therapy; Methylene Blue; Fusarium keratoplasticum; Candida albicans

Financial support: Capes, FAPESP

P18 - Light post-transcriptionally controls gene expression in Metarhizium acridum

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Aims. To combine transcriptomics (mRNA-Seq) and TMT-based high throughput proteomics to study light response in the entomopathogenic fungus Metarhizium acridum.

Methods. Twenty-four-hour-old cultures grown in the dark were briefly exposed to visible light for 5 min and returned to dark conditions for different periods according to the technique employed: 0, 10, 25, 55, and 115 min for mRNA-Seq; and 10, 25, 55, 115, and 235 min for proteomics. Cultures kept in the dark (no light exposure) were used as controls (DD). Fold change was calculated relative to DD for each time point. Transcripts and proteins were considered regulated if their abundance changed at least two-fold relative to DD.

Results. Light exposure resulted in 1128 genes (11.3% of the genome) being regulated at the transcript level. The number of proteins changing in abundance was only 57. Combining the two datasets, only 34 proteins were regulated both at the transcript and the protein levels. Log2-log2 correlation curves revealed that transcript increase at 0-25 min after light exposure best correlated with protein change at 1-2h. Because only 34 transcripts/proteins were commonly regulated in both datasets, we were left with 23 proteins that changed in abundance in the absence of mRNA regulation and also 1094 regulated transcripts for which there was no protein change. Some transcripts among these 1094 genes were up-regulated by as much as 18-fold and down-regulated by as much as 5-fold. Clustering these genes by molecular function revealed there is no functional category enriched.

Conclusion. Light regulates gene expression both transcriptionally and post-transcriptionally in M. acridum. Our results show that even when mRNA is greatly regulated by light there is no guarantee that protein levels will change and therefore proteomics should always be employed for a correct understanding of light regulation in organisms.

Keywords: Metarhizium; light; proteomics, transcriptomics; gene regulation.

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P19 - Unravelling the antioxidant and peroxidase function of thioredoxin-dependent peroxide reductase of the apicomplexan Neospora caninum

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The apicomplexan protozoan Neospora caninum, the etiological agent of neosporosis, is associated with neurological symptoms in dogs and abortion in cattle. This disease is worldwide spread and has no efficient treatment. The survival and replication of the parasite depends on an enzymatic antioxidant system defense for evading the oxidative stress inside the host cell. Among these enzymes, thioredoxin-dependent peroxide reductase (NcPrx) draw our attention due to a significant relative abundance in the proteome of Neospora caninum allied to the absence of literature. Therefore, we performed an initial characterization of the N. caninum antioxidant enzyme NcPrx. Recombinant NcPrx (rNcPrx) was expressed in Escherichia coli and analyzed by SDS-PAGE. The molecular weight of rNcPrx was in accordance with the predicted size 22 kDa. Purified rNcPrx1 was used to obtain mouse polyclonal anti-serum after four immunizations. In western blot analyses, anti-rNcPrx1 serum detected the recombinant forms of NcPrx1 and the native protein in the protein extract of N. caninum tachyzoite. Confocal immunofluorescence suggests mitochondrial localization of NcPrx1 in N. caninum tachyzoites. The antioxidant activity of rNcPrx was evaluated through mixed-function oxidation assay that determines the ability in protecting DNA from damage in oxidative conditions. rNcPrx showed antioxidant activity in concentration-dependent manner (610 µg/mL - 76,25 µg/mL), being gradually extinguished in lower rNcPrx concentrations (38,125 µg/mL). The peroxidase activity of rNcPrx1 in the presence of dithiothreitol (DTT) was evaluated through ferrothiocyanate system assay. rNcPrx1 exhibited a time and concentration dependent activity to reduce H2O2 in comparison to control (without enzyme, $p<0,05$). Our study has so far characterized NcPrx both via native enzyme detection and by demonstrating that recombinant NcPrx1 acts as functional enzyme of antioxidant system defense. These results enlarge the knowledge of Apicomplexan redox system and enables further investigations of drugs with unknown mechanisms of action, contributing for further research advances in neosporosis.

Keywords: Apicomplexa; Neospora caninum; Thioredoxin-dependent peroxide reductase; Peroxiredoxin; Antioxidant; Recombinant protein.

Financial Support: CAPES

P20 - Study of resistance, virulence and epidemiological profile of Escherichia coli isolated from environment and animals

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Aims: This study aims to characterize 100 isolates of Escherichia coli obtained from the environment and animals regarding antimicrobial resistance, virulence, clonal and epidemiological relationship and the presence of plasmids.

Methods: The isolates were obtained from soil and water samples and different animals. Then, the isolates were identified by 16S rRNA gene sequencing. The antimicrobial resistance profile was determined by disk diffusion method. The resistance and virulence genes, integrons and plasmids were detected by PCR. Epidemiological analyzes will be performed using the Pulsed-field Gel Electrophoresis (PFGE) and Multilocus Sequence Typing (MLST) techniques. The complete genome sequencing will be performed for some isolates using the Illumina MiSeq Platform.

Results: A total of 97 isolates were obtained and 43 of them were analyzed for antimicrobial resistance profile, being 41 of them classified as MDR. According to resistance profile, different plasmid resistance genes were investigated. The tetB gene was the most prevalent, being detected in 31 isolates, followed by tetA (29), blaSHV (19), blaOXA-1-like (18), blaCMY (17), sul2 (13), aac(6')-Ib (9), blaCTX-M-Gp9 (7), tetD (6), aph(3')-Ia (4), aac(3')-IIa (2), sul3 (3), qnrS (2), tetC (1), sul1 (1), qnrB (1) and oqxB (1). The integron class 1 was detected in 17 isolates. The virulence genes were investigated in 17 isolates and a total of 16 genes were found, being stx1 (7), ehxA (5), eaeA (2) and stx2 (2). These isolates were classified into two different pathotypes, with six STEC and two EHEC. Plasmid typing was performed on 32 isolates and the most prevalent plasmid family was I1 (26), followed by F (22), FIB (18), Y (17), HI1 (11), ColE-like (13), N (9), FIA (5), T (4), K (3) and FIC (1).

Conclusion: To date, it is possible to observe a high level of antimicrobial resistance and a diversity of resistance genes and plasmids.

Keywords: Escherichia coli; antimicrobial resistance; virulence; epidemiology; plasmid.

Financial Support: FAPESP (grant number 2015/18990-2) and CAPES (fellowship grant number 88882.180855/2018-01).

P21 - Differential reconstitution of B-cell subsets in systemic sclerosis patients after autologous hematopoietic stem cell transplantation

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Introduction: Autologous hematopoietic stem cell transplantation (AHSCT) is currently an effective alternative therapeutic approach for patients with severe systemic sclerosis (SSc), although its immune mechanisms are still not completely understood. Aim: To evaluate the reconstitution of naive, memory, regulatory and exhausted B-cell subsets in SS patients following AHSCT. Methods: Peripheral blood samples were harvested from eighteen diffuse SSc patients before transplantation and at 30, 60, 120, 180 and 360 days post-AHSCT. The immunophenotype and IL-10 production of B-cell subsets was assessed by flow cytometry. Results: Compared to baseline, the frequency and absolute counts of total CD19+ B-cells showed a significant decrease at 30 days, followed by an increase at 360 days post-AHSCT. The CD19+CD24hiCD38hi regulatory B cell (Breg; transitional B-cell subset) significantly increased in frequency and absolute counts at 360 days ($P<0.05$), while CD19+CD24hiCD27+ Breg (B10 subset) transiently decreased in absolute levels at 30 days ($P<0.01$). The CD19+CD38hi27hi Breg (plasmablast subset) increased at 15 days in frequency and absolute numbers ($P<0.05$). The frequency of CD20+CD43+CD27+CD69- (B-1 subset) increased at 30 days, followed by a decrease at 60 to 360 days ($P<0.05$), while no changes were detected in absolute numbers. Naïve B-cells significantly decreased in frequency and absolute counts at 30 days ($P<0.001$), followed by an increase at 360 days ($P<0.05$). There was a transient increase of non-class-switched memory-B-cells (CD19+CD27+IgD+) frequency at 30 days, followed by an increase to 360 days. The mature class-switched memory-B-cells (CD19+CD27+IgD-) frequency decreased at 120 to 180 days ($P<0.05$). No changes were detected in CD19+CD27highIgD- plasma-cells. The frequency of CD19+PD1+ exhausted B-cells increased at 30 days ($P<0.05$). The frequency of IL-10 producing Bregs CD19+CD24hiCD38hi and CD19+CD24hiCD27+ increased at

180 to 360 days post-transplantation. Conclusion: SSc patients showed increases of Breg frequencies and/or absolute numbers after AHSCT as well as increase of IL-10 production, suggesting improvements of immunoregulatory mechanisms. Furthermore, following transplantation SSc patients have decreased memory-B-cells and increased naive-B-cells values, which might contribute to self-tolerance reestablishment and disease remission on these patients.

Keywords: Systemic Sclerosis; Autologous Hematopoietic Stem Cell Transplantation; B cell; Regulatory B-cell; Cellular therapy

Financial Support: FAPESP and CAPES

P22 - MicroRNA regulation in mesenchymal stromal cells of Philadelphia-negative classical myeloproliferative neoplasms

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Polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) constitute the Philadelphia-negative classical myeloproliferative neoplasms (MPN) and are stem cell-derived clonal disease characterized by myeloproliferation and apoptosis resistance. MPN pathogenesis has been associated to driving mutations as JAK2V617F and CALR and malignant transformation of bone marrow niche (BMN). Mesenchymal stromal cells are a key element of BMN acting as a hematopoiesis regulator, supporting and maintaining hematopoietic stem cells functions. Functional alterations and cytogenetic abnormalities on MSC of MPN patients have been associated with early senescence of MSC, bone marrow fibrosis and reduction of hematopoiesis support. Although, the epigenetic (microRNAs) regulation of BM-MSC from MPN patients and their role in BMN transformation in NMP have not been reported. Aims: to evaluate the expression of microRNAs related to apoptosis and tumorigenesis in MSC of patients with MPN and controls (CTRL). Subjects and Methods: Bone marrow MSC were isolated from 9 MPN patients (5 PV, 2 ET and 2 PMF; median age= 60 years; 6 men and 3 women) positive for JAK2V617F mutation and 5 healthy donors (median age= 38 years, 3 men and 2 women).This study was approved by ethics committee CEP/FCFRP (process number 408 – CAAE 55545716.6.0000.5403).

MSC were cultured in α-MEM medium (1% de penicillin/estreptomycin, 1% L-glutamine and 15% FBS) at 37ºC with 5% CO2. Immunophenotype characterization by flow cytometry and differentiation potential was performed. RNAs were extracted using Trizol™ reagent, the cDNA was synthesized using the High Capacity cDNA Reverse Transcriptionkit (Applied biosystems™). The miR-16, miR-21, miR-26a, miR-29c and miR-130b expression analysis was performed using TaqMan MicroRNA Assays® (Applied biosystems™), according to the manufacturer's protocol. The MCL1 and BAK1 expression analysis was performed using SYBR™ green (ThermoFisher Scientific). Results were expressed as relative units of expression (RUE). Data were analyzed by the one-tailed Student's t-test (Prism5, GraphPad Software Inc. CA). Values of $p<0.05$ were considered statistically significant. Results: The miR-29c expression was lower in MSC of NMP patients in comparison to CTRL ($p=0.0453$). No statistical difference was observed in the expression of miR-16, miR-21, miR-26a and miR-130b between MPN and CTRL ($p>0.05$). The expression of predict targets for miR-29c, the anti-apoptotic gene MCL1 and the pro-apoptotic gene BAK1 was not statistically different between MPN and CTRL. Conclusions: Despite the MCL1 and BAK1 gene expression was not altered by the deregulation of miR-29c in MPN, there are still others predict target genes related to apoptosis to be evaluated, as BCL2 and BIRC2. Also, in some cases microRNAs regulate expression of mRNAs at post-transcriptional level, affecting only proteins levels. The lower expression of miR-29c founded in MSC of MPN patients may indicate that tumor HSC may regulates MSC survival and this could be essential for the diseases pathogenesis, specially in regards of BMN malignant transformation. Further investigations are necessary in order to better understand whether miR-29c deregulation contributes to MSC alteration.

Keywords: mesenchymal stromal cells; myeloproliferative neosplasms; microRNAs; bone marrow niche.

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P23 - Characterization of the pathogenic potential of Shigella flexneri strains isolated for 34 years in Brazil

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According to the World Health Organization (WHO) every year around 1.1 million deaths from shigellosis occurs worldwide. In developing countries like Brazil, endemic shigellosis has been mainly caused by Shigella. flexneri and Shigella sonnei. The pathogenicity of Shigella is associated with several genes located on the chromosome or on the virulence plasmid pINV of 220 Kb. The ipaH gene is present in the chromosome and in the virulence plasmid and is associated with the invasion of bacteria into host cells. The plasmidial genes ipaBCD and ial are responsible to activate the type III secretion system and the invasion process, respectively. The sepA gene encodes proteases. The sen (ShET2) produces enteroxins. Among the chromosomal genes: sigA encodes proteases responsible for intensifying intestinal fluid accumulation; iuc acts in the production of aerobactin; the set1A and set1B (ShET1) encode enterotoxins. The sat gene produces a serine protease autotransporter toxins (SPATEs). The aim of this study was to analyze the pathogenic potential of S. flexneri strains isolated for more than three decades in different States of Brazil. A total of 137 S. flexneri strains isolated from human diarrheal feces from 1983 to 2017 from Amapá, Bahia, Ceará, Maranhão, Minas Gerais, Pará, Pernambuco, Rio de Janeiro, Rio Grande do Norte, Rio Grande do Sul, São Paulo and Sergipe States were studied. The frequency of 10 virulence genes was verified by PCR. All the strains studied presented the ipaH genes. The ipaBCD, ial, sepA and sen genes were detected in 33 (24 %), 16 (12%), 42 (30 %) and 98 (71%) strains, respectively. The sigA, iuc, set1A, set1B and sat genes were detected in 114 (83%), 133 (97%), 53 (38%), 75 (55%) and 81 (59%) strains, respectively. All the strains studied presented one or more virulence genes. The results obtained highlighted the high pathogenic potential of the S. flexneri strains isolated in Brazil studied.

Keywords: Shigella flexneri, virulence genes, PCR, pathogenic potential.

Financial Support: CAPES e FAPESP"

P24 - SPHINGOSINE KINASES IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Head and neck squamous cell carcinoma (HNSCC) can arise from oral and nasal cavity, salivary glands, pharynx, larynx, and paranasal sinuses. HNSCC is the sixth most common type of cancer and eighth cause of death worldwide. Several studies have appointed some sphingolipids as “bioactive lipids” and as key regulators in different cellular processes. The balance between ceramide and sphingosine, for example, is known to control apoptosis and cellular senescence, while sphingosine-1-phosphate (S1P) plays a crucial role in cell survival and migration. The level of S1P can be regulated by sphingosine kinases (SphK1 and SphK2). Albeit the action of SphK1 has been implicated in tumor growth and cell transformation in HNSCC, the role of SphK2 is unknown. In the present work, our goal is to understand how SphK1, SphK2 and sphingolipids participate in HNSCC, looking for potential therapeutic targets and strategies against cancer. The cellular levels of SphK1 and SphK2 in HNSCC and non-tumor keratinocyte (NOK) cell lineages were determined for proteins by Western blot (WB) and for mRNA levels by real-time PCR (qRT-PCR). The cellular distribution of the SphKs were analyzed by immunofluorescence using anti-SphK1 and anti-SphK2. Knockdown of SphK1 and SphK2 proteins in HNSCC cell lineages was performed by RNA interference (shRNA), while their overexpressions were obtained in HNSCC cell lineages and NOK cell by using expression vectors containing SPHK1 and SPHK2 cDNAs. Our results showed alterations in several signaling pathways when both SphKs were overexpressed or knocked down in all cell lineages, affecting different cellular parameters such as histone acetylation, proliferation, clonogenicity and cell cycle progression. In conclusion, we can propose that both SphKs are important in HNSCC.

Keywords: oral cancer, sphingolipids, sphingosine kinase, cell signaling.

Financial support: FAPESP, CEPID/CTC-FAPESP, CAPES and CNPq.

P25 - Heterologous expression of a mitochondrial nicotinamide adenine dinucleotide transporter (Ndt1) from Aspergillus fumigatus in HEK293 cells with citrin deficiency

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Redox balance in mammalian mitochondria is performed by the aspartate-glutamate carrier (AGC), which is the main mechanism for the movement of reducing equivalents in the form of NADH.Type II citrullinemia (CTLN2) is an adult-onset autosomal recessive disease caused by mutations in SLC25A13 gene, and that coding citrin. Citrin is an isoform of AGC and catalyzes the transport of cytosolic glutamate through exchange with mitochondrial aspartate. It will be used in the urea cycle. CTLN2 causes urea cycle deficiency and hyperammonemia. Citrin deficiency cause an increase in the cytosolic NADH/NAD+ ratio. The increase in this ratio inhibits glycolysis and gluconeogenesis. The development an in vitro CTLN2 model is important for new studies about the disease mechanism and new therapies. Heterologous expression of proteins from different organisms has been used to recover some mitochondrial diseases. Biochemical and molecular studies in our laboratory demonstrated the presence of a mitochondrial nicotinamide adenine dinucleotide transporter (Ndt1) in Aspergillus fumigatus. Ndt1 protein performs cytosolic NAD+ transport to the mitochondria matrix, thus being an important protein to keep the redox balance in A. fumigatus. Thus, the aim of this work was to obtain a line of HEK293 mammalian cells with knockdown for the SLC25A13 gene, an in vitro CTLN2 model and the heterologous expression of the Ndt1 protein as a form of metabolism recovery. Cells with citrin knockdown showed an increase of cytosolic NADH/NAD+ ratio, reduction of glycolysis, reduction of urea concentration, and hyperammonemia. Expression of Ndt1 protein was able to reduce cytosolic NADH/NAD+ ratio and recovered the glycolytic activity. However, Ndt1 protein was not able to increase the urea concentration and reduce hyperammonemia caused by CTLN2. Thus, our results suggest that expression of Ndt1 protein in mammal cells recovers the mitochondrial metabolism and glycolytic activity in CTLN2 cells but does not improve urea cycle and hyperammonemia.

Keywords: Citrullinemia, Mitochondria, Citrin, Mitochondrial nicotinamide adenine dinucleotide transporter, Aspergillus fumigatus.

Financial Support: FAPESP; CNPq and CAPES

P26 - STUDY OF THE EFFECT OF THE COMBINATION OF FTY720 AND CISPLATIN IN CANCER STEM CELLS FROM SQUAMOUS CELL CARCINOMA OF THE ORAL CAVITY

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Oral cavity cancer is the most common type of head and neck cancer with the worst prognosis. This, due to late diagnosis, low therapeutic response, relapse in the primary site and aggressive metastasis, or development of second primary tumors. Cancer stem cells (CSC) are cells that show similar characteristics to stem cells; possess high tumorigenicity and migration capacities, which are important to promote field cancerization, resistance to therapy and recurrence that significantly reduces the survival of the patient with oral squamous cell carcinoma (OSCC). Based on the above, it is evident the need to define new therapeutic strategies against OSCC that eliminate CSC. Recently, it has been suggested that the combination FTY720 and cisplatin has antitumor effect, but their action against CSC has not been determined. In this way, it is proposed in this study to determine the effect of the combination of two drugs, FTY720 and cisplatin, on OSCC and CSC. For this purpose, flow cytometry assays will be carried out to characterize the CSC subpopulation as well as spheroid formation (3D culture) and clonogenic assays in the presence and absence of the drugs. If the combination of drugs is effective in reducing CSC in vitro, the xenograft tumor formation assay of OSCC in nude mice and CSC analysis of tumors will be carried out, as a proof of concept in vivo. It is expected that at the end of this study it will be possible to propose a new therapeutic strategy with high efficiency against CSC in oral squamous cell cancer, and in this way, have a therapy that eliminates the recurrence of the disease in the future.

Keywords: oral squamous cell cancer, cancer stem cells, FTY720, recurrence.

Financial support: CAPES, FAPESP and CNPq.

P27 - The role of the CD14 receptor in the metabolic regulation of macrophages stimulated with the venom of the scorpion Tityus serrulatus.

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The venom of Tityus serrulatus scorpion (TsV) is constituted by diverse compounds, which interact with innate immune cells and triggers signaling cascades that culminate on the inflammatory process. Envenoming induces alterations in several organs, deregulate ionic channels and can be fatal. Recent studies from our group described that macrophages are one of the main innate immune cells to recognize TsV. This process is performed by molecular pattern recognition receptors, highlighting the CD14 receptor present in these cell surfaces, and when activated, macrophages produce pro-inflammatory mediators. Recent observations implicate CD14 on a general metabolic regulation, since CD14-deficient animals do not become obese and they do not develop insulin resistance or cardiac complications associated to obesity. However, the molecular mechanisms by which CD14 coordinates the metabolic activity of immune system cells have not been elucidated. In this project, we will investigate the role of CD14 on the metabolism of bone marrow derived macrophages and peritoneal macrophages of C57BL/6 and CD14-/- after the stimulation with TsV or LPS. We will evaluate metabolic alterations resulting from the two stimuli, which will be compared between bone marrow derived macrophages or resident on the peritoneal cavity. For this purpose, we will use high resolution mass spectrometry coupled with liquid chromatography, a state-of-art technology on the field of metabolomics and systems biology. This project has great potential to reveal metabolic pathways and molecular mechanisms induced by TsV, how these processes are regulated by CD14 receptor, and for the design of new therapeutic strategies on the envenoming by TsV and related diseases/conditions.

Keywords: macrophage, Tityus serrulatus, metabolomics, venom.

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P28 - The role of pituitary hormones during the accute phase of Chagas Disease in Wistar rats.

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Trypanosoma cruzi (T. cruzi), the etiological agent of Chagas Disease (CD), can be found in the adrenal glands, while the amplification of one of its specific genes demonstrated that its product was found both in the adrenals and in the pituitary glands of infected animals. However, not many studies investigated changes in the secretion of pituitary hormone and its participation during the response process to the T. cruzi.

Aim: The objective of this work is to study some neuroimmunoendocrine parameters of male adult Wistar rats during the acute phase of CD.

Methods: We first analyzed the secretion of the pituitary hormones vasopressin (AVP) and oxytocin (OT) with Radioimmunoassay during the acute phase of the disease. The Nitric Oxide (NO) quantification in the cerebrospinal fluid and serum was in accordance to the Griess Reaction technique. To understand possible changes in hypothalamus-pituitary-adrenal (HPA) axis responsiveness to specific stimuli, we induced osmotic challenge due dehydration to young male Wistar rats with CD, as a way to investigate possible alterations of secretion of AVP and OT. After this, we evaluate the effects of OT (3 IU/Kg administered subcutaneously) treatment on NO, IFN-γ and IL-2.

Results: while systemic and cerebrospinal fluid NO increases in the early stage of the disease, the AVP and OT hormones are below basal levels. However, the groups of animals subjected to a strong stimulus for the secretion of these hormones, through osmotic challenge after dehydration, normally respond with hormonal secretion. While infection causes changes in IFN-γ and NO, treatment with OT increases systemic NO and IL-2. Conclusion: The acute phase of CD causes changes in the HPA axis without altering some physiological responses in infected animals. Further studies may clarify the role of NO and OT changes during CD.

Keywords: Hypothalamus; Neurohypophysis; ELISA; Osmolality;

Financial Support: CNPq; FAPESP

P29 - ASCORBIC ACID ASSOCIATED WITH LOW DOSE OF BENZNIDAZOLE IMPROVES THE THERAPY OF CHAGAS DISEASE: A DRUG-SPARING REGIMEN.

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Despite being one of the highest impact infectious diseases in the Americas, Chagas' disease is treated in restricted cases. This fact is due to the lack of efficacy during a chronic phase and the non-specificity of the biological targets of Benznidazole (BZ), which confers numerous side effects. Since it is the only drug available for therapy in several countries, the need to develop new therapeutic alternatives is triggered. In this sense, the research of drug-sparing regimens is increasing, aiming to reduce the dose and/or the duration of BZ therapy. In addition, associating BZ with antioxidant compounds such as ascorbic acid (AA) has as purpose the neutralization of oxidative stress, important in the development of the pathogenic process. In this study, BZ (25mg/kg/day) was used as a reduced dose and AA was associated in lower (aa-7.14mg/kg/day) and higher (AA-88.1mg/kg/day) dosages. Female Swiss mice (6-weeks-old) infected with T. cruzi Y strain were orally treated for 15 days and had some parameters evaluated after this period. A greater reduction in parasitemia was observed in the BZ+AA group, when compared to the drug alone. Moreover BZ+AA decreased the heart weight/body weight ratio and this reduced BZ dose (associated with ascorbic acid or not) prevented weight loss at the end of the experiment when compared to the infected control (IC). Biochemical parameters showed no change in creatinine levels and the dosage of transaminases indicated that the clinical dose BZ (100 mg/kg/day) increased the levels of AST while the reduced dose proposed in this study did not alter this biomarker. These results indicate that there are benefits in reducing the dose of the BZ and when associated with AA the trypanocidal activity can be improved.

KEYWORDS: Ascorbic acid; Benznidazole; Chagas' disease, Drug-sparing regimens

Financial Support: CAPES

P30 - Function and metabolic profile of platelets on the experimental infection by Achromobacter xylosoxidans”

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Platelets are anucleate blood elements that have considerable role in modulating hemostasis. Recent studies indicate that platelets are essential regulators of the immune system and exhibit a fundamental role in infections. Platelets store high amounts of molecules that promote direct pathogen elimination or modulate effector activities of leukocytes. Recently, we have characterized lung experimental acute infection by Achromobacter xylosoxidans, in which we showed the essential function of the innate immune system for infection control. These gram negatives bacili are normally found in the environment and are opportunistic pathogens infecting persons with cystic fibrosis (CF), tumors and immunodeficiencies. However, the molecular mechanisms that drive the host response against A. Xylosoxidans infections are unknown. Therefore, we hypothesized that platelets contribute to host response against lung infections by bacteria. We also speculate that the metabolic profile of platelets is tightly associated with their effectors activities. In this work, we propose to use classical immunological techniques combined with strategies of systems biology (lipidomics and metabolomics) that will enable us to evaluate: 1) platelet interaction and antimicrobial activities against A. Xylosoxidans; 2) platelet activation and production of mediators in response to bacteria and 3) identify the metabolomic profile of the interaction between platelets and A. Xylosoxidans and associations with produced inflammatory mediators. Therefore, we will demonstrate that platelets exert a significant role during A. xylosoxidans infection and elucidate the metabolic interaction between pathogen and platelets.

Keywords: platelets, Achromobacter xylosoxidans, host response, immune system

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P31 - INCREASING CONIDIAL RESISTANCE TO ANTIMICROBIAL PHOTODYNAMIC TREATMENT WITH NEW METHYLENE BLUE IN PLANT-PATHOGENIC FUNGUS COLLETOTRICHUM ABSCISSUM

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Aim: To investigate the selection of strains of the plant-pathogenic fungus Colletotrichum abscissum resistant to antimicrobial photodynamic treatment (APDT). Methods: In a one-and-a-half-year-long experiment, we carried out sixty successive cycles of APDT of conidia of the Colletotrichum abscissum using the photosensitizer New Methylene Blue N (NMBN) combined with exposure to red light. The treated conidia of the fungus were then plated on potato dextrose agar medium. During subsequent incubation, the conidia borne on colonies from viable conidia (i.e., those which have survived the APDT) were pooled and submitted to the next cycle of treatment and selection. Results: We identified 12 carotenoids in conidia of C. absissum: (15Z) or (15'Z)-neurosporaxanthin; (13Z) or (13'Z)-neurosporaxanthin; (Z)-neurosporaxanthin; (all-E)-neurosporaxanthin; (15Z) or (15'Z)-neurosporaxanthin methyl ester; (13Z) or (13'Z)-neurosporaxanthin methyl ester; (Z)-neurosporaxanthin methyl ester; (all-E)-neurosporaxanthin methyl ester; (Z)-g-carotene; (all-E)-g-carotene; (all-E)-lycopene and torulene. For 11 out of 12 carotenoids identified, there was an increase in accumulation in the conidia throughout the successive cycles of APDT. The only exception was (all-E)-g-carotene. We also observed successive increases in conidia resistance to APDT throughout the experiment. At the end of the 60th selection-round, we compared the conidial survival between the initial isolate and the isolate selected after 60 selection cycles in three stress conditions, using fluences of 7,3 J cm-^{2}; 21,8 J cm-^{2} and 43,7 J cm-^{2} obtained after exposure to red light for 5, 15 and 30 minutes, respectively. It was found that conidial survival was 2.5 times higher than that of the initial isolate for the experimental condition of exposure to a fluence of 21,8 J cm-^{2}. Although the genetic basis of increased APDT tolerance and carotenoid production have yet to be determined, we believe that these two characteristics are correlated, since carotenoids quench singlet oxygen, the main reactive oxygen species produced during APDT with NMBN. Carotenoids in conidia have already been associated with increasing tolerance to APDT with phenothiazinium photosensitiziers in conidia of Neurospora crassa. Although somehow our results may cause concern to the APDT area, they cannot be considered completely unexpected, since there are other genera of plant-pathogenic fungi that are naturally resistant to photodynamic treatment. For

instance, Cercospora species produces the potent photosensitizier cercosporin, which is used to damage the issues of the host plant but are themselves resistant to its deleterious effects. Conclusions: For the conditions and fungal specie tested, we found development of resistance to APDT as increasing of conidial survival between the initial isolate and the isolate selected after 60 selection cycles.

Keywords: antimicrobial photodynamic treatment; Colletotrichum abscissum; resistance; increasing of conidial survival.

Financial Support: CAPES, FAPESP

P32 - Production of lentiviral vectors under scalable conditions for generation of CAR-T cells for cell therapy

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Studies with the use of modified T lymphocytes with Chemical Antigen Receptors (CAR) present expressive results in the treatment of leukemias and lymphomas. Lentivirus are widely used with vectors of gene modification in this type of therapy, with the reason of their advantages, such as cell genome stability, transduction efficiency and safety. Monolayer cultivation is limited because it does not respond to the demand for large-scale prodution of these vectors, requiring suspension cell culture for application in biorreactor. The aim of this study is to perform the production of lentiviral particle in scalable conditions and according to Good Manufacturing Practices, using serum-free media in suspension culture for the generation of CAR-T cells. Therefore, HEK293T cells adapted to serum-free suspension culture will be used to produce lentivirals particles expressing a synthetic anti-CD19 protein. Initially, small-scale experiments will be performed by transient transfection using PEI. In this step, parameters such as, use of additives to increase efficiency and ratio of plasmids and PEI transfection agent, will be evaluated and applicated in the production in stirred tank bioreactor. With this project we hope to develop an efficient, reproducible and economically viable platform for the production of lentiviral vectors that contributes significantly to the research related to cell immunotherapy.

Keywords: lentiviral vectors; CAR-T cells; HEK 293T; suspension culture; serum free media; bioreactor; immunotherapy

Financial support: CAPES and FAPESP

P33 - EVALUATION OF THE BACTERICIDAL EFFECT OF GALECTIN-4 IN Escherichia coli EXPRESSING TYPE B BLOOD GROUP ANTIGEN

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Background: Galectins are soluble proteins that recognize glycans containing β-galactosides residues and participate in many cellular and molecular processes related to infectious diseases. Along this line, galectin-4 has been reported to recognize and kill E. coli O86, which expresses O antigen of LPS with similar epitope to human blood group B erythrocyte (BGB+ E. coli). Although this bactericidal effect is known to be fast and carbohydrate recognition-dependent, little is known about its action mechanism. Aims: to evaluate the bactericidal impact of galectin-4 in E. coli O86. Methods: Human recombinant galectin-3 and -4 were produced in plasmid-transfected E. coli and purified using affinity-chromatography sepharose-lactose column. The preservation measurement of the lectin activity of purified galectins was assessed through hemagglutination assay. The bactericidal effects of purified galectins were evaluated by counting the Culture Forming Units (CFU) of BGB+ E. coli and mutant ΔwaaL BGB- E. coli. The galectin-treated E. coli strains real-time oxygen consumption test was evaluated by Oroboros® Respirometric Assay. Results: The purified galectin-3 and -4 preparations were homogenous with apparent molecular weight of ~30 and ~35 kDa, respectively. The purified proteins were able to promote efficient erythrocyte agglutination, which indicates that the purified galectins were fully active. Galectin-4, and not galectin-3, was able to kill BGB+ E. coli and not the BGB- E. coli, as expected. Curiously, in the respirometric assay, galectin-4-treated BGB+ E. coli did not interrupt oxygen consumption, even in lethal concentration of the galectin. Interestingly, post-technical analysis of this experiment showed reduction of CFU and an agglutination phenotype of the galectin-treated bacteria. Conclusions: human recombinant galectin-4 presented bactericidal effect to E. coli O86 with unexpected properties toward the bacteria oxygen consumption.

Keywords: galectins; E. coli O86; respirometry; LPS

Financial support: CAPES

P34 - CHARACTERIZATION OF EXPANDED UMBILICAL CORD DERIVED-MESENCHYMAL STROMAL CELLS IN STIRRED TANK BIOREACTOR CONDITIONED WITH HYPOXIA

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Multipotent Mesenchymal Stromal Cells (MSC) have been extensively investigated in pre-clinical and clinical trials for cell-based therapies because of their remarkable anti-inflammatory, immunomodulatory and regenerative properties. However, some limitations, such as cell senescence by excessive in vitro expansion, reduction or inconsistency of the therapeutic potential and low survival of transplanted cells, require immediate search for priming strategies and new approaches GMP-compliant expansion to produce robust and functional MSCs for therapies. In this study, we established a scalable bioprocess to expand MSC from human umbilical cord (hUC-MSC) conditioned with hypoxia under xenoantigen-free conditions. hUC-MSCs were expanded in stirred-tank bioreactor coupled to human collagen coated microcarriers using AB human serum and conditioned with hypoxia (dissolved oxygen concentration maintained to 5%). Subsequently, were characterized by immunophenotyping and differentiation and immunosuppressive potentials. To access in vitro immunosuppressive function, hUC-MSC post-expansion were co-cultured, in different concentrations, with peripheral blood mononuclear cells stained with CFSE (3uM) and stimulated with DynabeadsTM activator (1:1). hUC-MSC expanded on 4-hour adhesion phase and intermittent shaking, showed significant cell expansion and presented excellent rate of cellular recovery. The established protocol enabled the production of 1,36 (±0,01) × 105 cells/mL after five days of culture, corresponding to a fold expansion of 6,11 (±0,63) based on the percentage of adherent cell to microcarriers (56,25 ±6,25%), similar normal DO (20%) conditions. Regarding immunophenotypic analysis, decreased CD105 expression post-expansion was observed.

Multipotential capacity to differentiate into adipogenic, osteogenic and chondrogenic lineages was retained. Expanded MSC primed with hypoxia showed elevated immunosuppressive potential, able to inhibit T CD8+ cell proliferation in the different ratios. These results represent an important step toward the establishment of a GMP-compliant large-scale production system for hypoxia-primed hUC-MSCs. New experiments are needed to evaluate the effect of priming with hypoxia to

immunomodulatory and regenerative in vivo potential.

Key Words: mesenchymal stromal cells; cell therapy; hypoxia

Financial support: FAPESP, CAPES

P35 - Study of possible antiparasitic effects of 1,6-diphenyl-1H-pyrazolo[3,4-b]pyridine derivatives in in vitro and in vivo models of infection by Leishmania braziliensis and Leishmania infantum

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Leishmaniasis are caused by the protozoan Leishmania and are considered one of the neglected tropical diseases, reaching mainly poor and less favored regions. In Brazil, cutaneous leishmaniasis (CL) caused by Leishmania braziliensis, is caracterized by ulcers with raised borders on skin. The visceral leishmaniasis (VL), the most severe form of the disease, is caused by Leishmania infantum and exhibits a systemic profile. The current therapy of these diseases forms are not considered effective, for being highly toxic, with low efficiency, high cost, long treatment period and the emergence of cases of resistance to some used drugs. The current search for new compounds has focused in the synthesis of novel molecules for medicinal chemistry purposes. Within this context, the modifications of the 1,6-diphenyl-1H-pyrazolo[3,4-b]pyridine scaffold can exert potent and selective actions at diverse targets. In this regard, our aim is to evaluate the action of the 1,6-diphenyl-1H-pyrazolo[3,4-b]pyridine derivatives in in vitro and in vivo models of L. braziliensis and L. infantum infections. For this, J774 cells will be infected with the parasites in vitro and the leishmanicidal activity, cytotoxicity and immunomodulation caused by these compounds will be evaluated. In in vivo assay, Balb/c mice will be infected and treated by gavage with the compounds using doses determined in in vitro assays. In the end of treatment, animals will be euthanized and the parasitic load, immunological parameters and toxical effects will be analyzed. Thus, we hope that the present project may be able to provide new therapeutic alternatives for the treatment of LC and LV.

Keywords: Leishmania braziliensis; Leishmania infantum; drugs; leishmanicidal effects

P36 - Effects of extract and fractions of Gigartina skottsbergii and extracts of Curvularia lunata on the production of reactive oxygen species by neutrophils

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Aim: To evaluate the effect of the seaweed Gigartina skottsbergii and the endophytic fungus Curvularia lunata on the production of reactive oxygen species by neutrophils. Methods: Extracts and fractions of G. skottisbergii and C. lunata were obtained by addition of different solvents (n-hexane, ethyl acetate and methanol), filtrated, concentrated under reduced pressure and analyzed by high-performance liquid chromatography with diode array detection (HPLC-DAD). C. lunata extracts were cultivated in artificial and natural sea water. Neutrophils were isolated from the whole blood of healthy subjects using the gelatin solution, treated with the extract and fractions and submitted to the chemiluminescence (CL) assay, using luminol as fluorescence probe. The CL assay was used for measurement of the production of the reactive oxygen species (ROS) by neutrophils that were stimulated with phorbol myristate acetate. Results: The results obtained by HPLC-DAD of the extract of the seaweed G. skottisbergii showed the presence of a major substance in 3.15 minutes, which presented maximum absorption of the ultraviolet radiation in 320nm. The chromatographic profile of the extract of C. lunata cultivated in natural sea water showed a greater diversity of metabolites when compared to the profile of the same fungus cultivated in artificial sea water, both extracts were obtained from liquid-liquid extraction with solvent ethyl acetate. The treatment with extract and fractions of the G. skottsbergii resulted in a decrease of the neutrophils' ROS production. The extract of C. lunata, cultivated in artificial sea water, decreased the neutrophils' ROS production. Conclusion: These results suggest the potential to obtain molecules with biological activity on the oxidative metabolism of neutrophils from the alga G. skottsbergii and the fungus C. lunata.

Keywords: neutrophils; marine seaweed; endophytic fungi.

Financial Support: CAPES

P37 - Expression of Recombinant HIV and HTLV Proteins and Peptides in Heterologous Systems

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The HIV and HTLV infections compose a serious public health problem. The existence of accurate, fast and accessible diagnostic methods is important, since identifying the presence of these retroviruses allows a better approach in the treatment and control of infections. Despite the advances of researches in Brazil, diagnostic kits used in the country are mainly of foreign origin. The production of diagnostics systems using national technology would imply a considerable reduction of costs, and independence or smaller dependence of the external market, also leading to an improvement of the diagnosis techniques and the production process quality. The present project aims express in a Pichia pastoris system, recombinant proteins from the HIV-2 and HTLV-2 retroviruses, to include them in rapid diagnostic systems. The sequences to be expressed were choosed from analyzes of the genomes of these retroviruses in the National Center for Biotechnology Information data bank. These sequences were cloned in pPICzα-A. The constructions will be used to transform Pichia pastoris cells by electroporation. The recombinant clones will be selected by growth in medium containing Zeocin. Methanol will be used as expression inductor during 6 days, and aliquots will be collected each 24 hours. The collected material will be analyzed by SDS-PAGE and Western blot. The P. pastoris clone that shows the higher protein production will be selected to increase the scale of heterologous expression. The recombinant proteins will be purified by Immobilized Metal Affinity Chromatography using a nickel column and then will have their identities confirmed by mass spectrometry.

Keywords: recombinant proteins; HIV; HTLV; Pichia pastoris

Financial Support: CAPES

P38 - Metagenomic prospection of quorum sensing related bacteria during spontaneous cocoa beans fermentation

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Aims: The spontaneous cocoa beans fermentation is a non-standardized process that is carried out by the autochthonous microbiota from the cocoa fruit. They come in contact with the cocoa pulp when the fruits are cut, starting the fermentation process, which is marked by a relatively synchronized microbial succession of yeasts, lactic acid bacteria and acetic acid bacteria. Therefore, we sought to understand this microbial succession from the viewpoint of cell-cell communication, hypothesizing the presence of interspecific molecular signalling via Quorum Sensing (QS) acting as a phenomenon that drives population dynamics during the microbial succession. Methods: One spontaneous fermentation of cocoa beans was performed in the state of Bahia, Brazil. Temperature and moisture from the surrounding environment were measured and cocoa samples from fermentation were collected at the initial time (0h), 6h, 24h, 30h, 48h, 54h, 72h, 96h, 120h and 144h. Samples were taken from ca. 30 cm deep from the fermentation box at three different points. One aliquot of 25g-sample was used for isolation of yeasts, lactic acid bacteria and acetic bacteria. The remaining fermented mass was frozen for later metagenomics analysis. Results: Our preliminary results, appointed the presence of yeast, lactic acid bacteria and acetic acid bacteria widely reported in the literature, with emphasis on species considered dominant and / or important to determine good sensorial traits for cocoa flavour development, such as Pichia Kluyveri, Pichia kudriavzevii, Lactobacillus plantarum, Lactobacillus fermentum, Leuconostoc mesenteroides and Acetobacter tropicalis. These findings may corroborate to prospect key strains in the design of a future starter culture for the cocoa fermentation.

Key-words: Cocoa fermentation, Quorum Sensing, Cocoa metagenomics, bacterial signalling.

Financial support: FAPESP(fellowship number 2017/13759-6)

P39 - MOLECULAR IDENTIFICATION AND ANTIFUNGAL SUSCEPTIBILITY OF CRYPTOCOCCUS NEOFORMANS AND CRYPTOCOCCUS GATTII CLINICAL ISOLATES OF RIBEIRÃO PRETO – SP

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The genus Cryptococcus presents two main species that are pathogenic to humans, Cryptococcus neoformans and Cryptococcus gattii. The infection caused by these fungi may affect both immunocompromised and immunocompetent individuals. The aim of this work was to study the fungal clinical isolates of patients with cryptococcosis from Clinical Hospital of the Medical School of Ribeirão Preto - USP, isolated between 2012 and 2017. The molecular identification of these species was performed by conventional PCR with specific primers to differentiate C. neoformans and C. gattii. Additionally, PCR – Restriction Fragment Length Polymorphism (RFLP) was performed to establish the Cryptococcus spp. molecular types through the amplification of the URA5 gene. The sequencing of the Internal Transcribed Spacer (ITS) of ribosomal DNA was used to confirm the clinical isolates of C. gattii. All clinical isolates were submitted to in vitro antifungal susceptibility test, by the broth microdilution method based on the M27-A3 protocol of the Clinical and Laboratory Standard Institute (CLSI). The tested antifungal agents were amphotericin B, itraconazole, voriconazole, fluconazole, and 5-flucytosine. Of a total of 111 clinical isolates, 98 (88%) were identified as C. neoformans, in which 91 isolates are molecular type VNI, 6 VNII, and 1 VNIII. Thirteen (12%) clinical isolates were identified as C. gattii and all are molecular type VGII. In the antifungals tests, the azoles (fluconazole and itraconazole) presented clinical isolates with dose-dependent sensitivity (DDS), in which both species were observed and the molecular types that showed DDS were VNI and VGII. Additionally, DDS was also observed among clinical isolates from HIV-negative and HIV-positive patients. Therefore, in this work was observed that the majority of the clinical isolates are sensitive to the antifungals used in the clinic.

Keywords: Cryptococcus neoformans; Cryptococcus gattii; molecular identification; PCR-RFLP; in vitro susceptibility.

Financial Support: CAPES, CNPq and FAPESP

P40 - ANTITUMORAL ACTIVITY OF BTHTX-I AND BTHTX-II FROM BOTHROPS JARARACUSSU ON HUMAN BREAST CANCER CELL LINES

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Breast cancer is the type with most incidence and mortality rate in women worldwide. Its therapy is defined according to the mammary carcinoma subtype, however tumors generally present resistance to the conventional treatments, creating a demand for new therapeutic approaches for this neoplasm. In this study, we have presented the effects of Bthtx-I and Bthtx-II toxins from Bothrops jararacussu on human breast cancer cell lines MCF7 (Luminal), SKBR3 (Overexpressing HER-2) and MDAMB231 (Triple Negative), in which it was evaluated cell viability when cells were treated with the toxins through MTT technique, cell death induction by apoptosis, necrosis and autophagy through flow cytometry and/or Western Blotting and cytotoxicity to cancer stem-cells (CSCs). Therefore, results have showed that both toxins at concentration of 102µg/mL decreased cell viability of all tumor cells studied. Besides, flow cytometry has indicated a higher apoptosis induction of MCF7 and SKBR3 cells when treated with Bthtx-I that when treated with Bthtx-II. Moreover, when Bthtx-I treatments were better explored, it was noticed a greater expression of Caspase 3 and Caspase on MCF7 cells, confirming a higher apoptosis induction by extrinsic pathway, as well as an increase of Beclin-1 protein expression, indicating cell death induction through autophagy. Further, Bthtx-I treatment has changed the labeling pattern of CSCs on MDAMB231 cells from CD44+/CD24- to CD44+/CD24-/low, causing cell death, although neither MCF7 nor SKBR3 cells have presented the peculiar staining pattern for CSCs. Thus, the present findings suggest that Bthtx-I and Bthtx-II are efficient in causing cell death to breast cancer cell lines, mainly by apoptosis and autophagy induction, of which Bthtx-I have shown to be a promising therapeutic approach for in vivo testing of breast neoplasm.

Key words: toxins, breast cancer, apoptosis, autophagy, cancer stem-cells.

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P41 - Hypothyroidism depresses immune response in Chagas disease.

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Hypothyroidism is a common endocrine disorder characterized by low or non-existent production of T3 and T4 hormones (TH) by the thyroid gland. TH is essential for human and animal development, growth, and function of multiple organs. Experimental and clinical studies have shown that unbalance in the production of thyroid hormones are involved to immune response failure, which can promote susceptibility to infections. Little is known about the association between hypothyroidism and neglected tropic diseases. In this context, our objective was to understand the impact of hypothyroidism on the model of Chagas disease. For this, rats were rendered hypothyroid after 21 days of treatment with 0.02% methimazole (wt/vol) in the drinking water and subsequently infected with T. cruzi intraperitoneally. On the 11th day of infection, the animals were euthanized and the immunological parameters investigated. Interestingly, we observed that hypothyroidism in chagasic disease induces late death of thymocytes, which impairs lymphocyte maturation. In addition, production of IL-17A in the plasma of animals was down regulated compared to control group. We found that the association of hypothyroidism and Chagas disease affects the migration of macrophages and B cells to the spleen. In conclusion, our data suggest that hypothyroidism promotes susceptibility in Chagas disease.

Keyword: Hypothyroidism, immune response, Chagas disease

Financial support: FAPESP, CAPES and CNPq

P42 - ENZYMATIC CHARACTERIZATION OF SUPEROXIDE DISMUTASE 2 OF Neospora caninum AND INVESTIGATION OF ITS ROLE AS A TARGET OF PHENOTHIAZINES DYES

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Neosporosis, a disease related to abortions in cattle, is caused by the apicomplexan protozoan named Neospora caninum. The antioxidant defense system of intracellular parasites such as N. caninum is composed of enzymes, where one of the most prominent is the superoxide dismutase (SOD). The role of SOD in invasion and survival of N. caninum inside the host cell remains to be elucidated. Therefore, this project aims to identify and functionally characterize the enzyme SOD2 (NcLIV_058830) of N. caninum. In addition, the activity of this recombinant protein under the action of phenothiazine dyes (methylene blue and derivatives) will be investigated. In order to achieve these objectives, cloning and expression of the recombinant NcSOD2 protein will be performed and polyclonal antibodies obtained. The immune recognition pattern of NcSOD2 will be investigated by western blot and ELISA, followed by mass spectrometry identification. The native NcSOD2 protein will be localized in the parasite by confocal immunofluorescence. The functional characterization of NcSOD2 will be determined by enzymatic and biochemical assays. The effects of phenothiazine dyes under the dark or the light on N. caninum invasion will be evaluated as well as the role of rNcSOD2 in these conditions. This project intends to reveal novel features of the N. caninum redox system through the NcSOD2 enzyme, enriching the knowledge towards the antioxidant system of the parasite and the role of this enzyme as an eventual target of the phenothiazine dyes.

Keywords: Apicomplexa, Neospora caninum; Superoxide Dismutase 2; Phenothiazine dyes

Financial Support: CAPES

P43 - Architecture and cellular composition of hematopoietic, thymic, lymphonodal niches of sickle cell anemia mouse model

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Sickle cell anemia (SCA) is one of the most common monogenic diseases in the world, which have a significant social impact. This syndrome is caused by a point mutation on the beta-globin chain gene, leading to physicochemical changes in the hemoglobin (Hb) molecule, resulting in abnormal hemoglobin named HbS. The vaso-occlusion episodes are the major clinical feature of SCA, which triggers acute pain crisis, chronic inflammation, high susceptibility to infections, progressive multiple organs injuries, and many others clinical complications. Although many organs are affected in SCA, there is no data available about how the disease affects the primary lymphoid (thymus and bone marrow) and secondary (lymph nodes and spleen) organs, and consequently the developmentof the T and B lymphoid lineages. Aim: To evaluate the architecture and cellular composition of hematopoietic, thymic, lymphonodal niches of sickle cell anemia. Methods: Townes mice (healthy and homozygous for HbS) will be studied at different stages of development (neonates, 6 weeks old, 12 weeks old), to evaluate the following aspects: a, size and weight of thymus, spleen, and lymph nodes; b, tissue architecture of primary and secondary lymphoid organs by histological analysis; c, cellular composition of hematopoietic/thymic/lymphonodal niches by confocal immunofluorescence analysis; d, immunophenotyping of developmental stages of B cells and other immune-cell types in the bone marrow; e, immunophenotyping of developmental stages of thymocytes, chemokine receptor expression and apoptosis quantification by flow cytometry,andthymocytemigration capacity; f, immunophenotyping of peripheral T, B and other immune cell subpopulations in lymph nodes and spleen by flowcytometry; g, serum cytokines and chemokines quantification by multiplex assay. Conclusion: This study will hopefully elucidate the mechanisms associated with the immune deregulation in SCA and could support the development of new strategies for prevention and treatment of infections in SCA patients.

Keywords: Sickle cell anemia;infections; primary lymphoid organs; secondary lymphoid organs; immune cells; immune deregulation

Financial Support: CAPES

P44 - PROFILE OF SPHINGOLIPIDS IN HEAD AND NECK CANCER.

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Sphingolipids are a class of essential lipids in eukaryotes not only by composing the plasma membrane but because they are bioactive molecules. Thus, they are involved in the regulation of many cellular processes and different types of signaling. Among the diversity of pathways that sphingolipids are associated with are included cell proliferation, apoptosis, senescence, angiogenesis, endocytosis, transport, migration, and inflammation. Among the main bioactive sphingolipids are ceramide and sphingosine which are molecules responsible for growth control, cell proliferation and survival. Another important sphingolipid is sphingosine-1-phosphate is a pro-survival molecule, acting in the autocrine, paracrine and / or endocrine way in cell proliferation, adhesion, motility, angiogenesis and inflammation. Because of the large number of pathways that these molecules regulate the imbalance in metabolism triggers a series of pathologies, such as cancer. In head and neck cancer the reduction in ceramide levels was associated with tumor progression and worse prognosis, but the other sphingolipids were not evaluated. Based on the potential impact of sphingolipid levels on cancer, we propose in this study to determine the profile of different sphingolipids in samples from patients with head and neck cancer (HNC) to evaluate possible associations with clinical and pathological parameters. Serum and / or blood samples as well as tumor fragments from patients with HNC will be used to extract sphingolipids. The determination of the sphingolipids will be done by the technique of mass spectrometry. The data will be submitted to statistical analysis to determine the significant associations between the different clinical and pathological parameters and sphingolipids. This study will improve our understanding on sphingolipids in head and neck cancer and their potential applications as biomarker and therapeutic targets.

Keywords: head and neck cancer, sphingolipids, mass spectrometry.

Financial support: FAPESP, CEPID, CAPES and CNPq.

P45 - Evaluation of the Trypanosoma cruzi Mucin's Synthetic Glycopeptides Potential Protective Activity and its Antibodies in Experimental Chagas Disease

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Introduction: The etiological agent of Chagas disease is the intracellular protozoan Trypanosoma cruzi (T.cruzi). This disease is endemic in South America and presents a global epidemiological profile, being 10 million people around the world affected by this disease. This parasitosis presents high morbidity and can be fatal. At the present moment, there is no vaccine for this disease and the treatment strategies and diagnostic approaches are not fully efficient to cope with this important parasitic infection. Objective: The goal of this work is to contribute to the development of new applicable therapeutic/diagnostic strategies for Chagas disease from the assessment of the Trypanosoma cruzi mucin's synthetic glycopeptides potential protective activity and its antibodies in experimental infection. Murine polyclonal and monoclonal antibodies for these glycopeptides will be obtained. The evaluation of the protector activity against T. cruzi infection will be made in mice treated with the glycopeptides or by the passive transference of its antibodies. Methods: The analysis of this protector effect will be made by the evaluation of the parasitaemia, determination of parasite load, heart histopathology analysis, leukocyte immunophenotyping, and cytokines quantification. It is understood that the development of this project may bring applicable data for the development of new terapeutic/diagnostic strategies for Chagas disease.

Keywords: Glycopeptides; Trypanosoma cruzi; Mucins; Antibodies; Chagas disease; Diagnostic.

Financial Support: CNPq and CAPES.

P46 - Selection and characterization of specific DNA aptamers to Trypanosona cruzi molecules for theranostic purposes in Chagas disease

Tálita Pollyanna Moreira dos Santos, Marcelo Dias Baruffi

The parasitic infection by the Trypanosoma cruzi triggers trypanosomiasis or also called Chagas disease. Estimation indicates about 8 to 10 million people infected by T. cruzi in South America. The mortality rate due to the complications caused by the infection represents about 6,000 deaths per year in Brazil. The identification of T. cruzi specific molecular targets may favor the development of novel therapeutical and diagnostic (Theranostic) strategies to fight Chagas disease with greater selectivity and lower cytotoxicity. Aptamers are small nucleotide sequences capable of binding to a wide variety of targets with high affinity, low immunogenicity, and high chemical modification ability in conjugation with other molecules. Galactose-alpha-1-3-galactose (alpha-gal), mucin-1, and trans-sialidase molecules are exclusively found on the surface of parasites and not in humans beings. Thus, the aim of this project is to identify and characterize DNA aptamers specific to the alpha-gal-1, mucin-1 and / or trans sialidase from trypomastigote form of T. cruzi using a synthetic library of aptamers. For this purpose, SELEX technique (Systematic Evolution of Ligands by EXponential Enrichment) will be used. The aptamer selection will happen in two steps. The first one consists of a challenge between the whole parasite and aptamer synthetic library, and the binding aptamers (parasite-Aps) will be collected and amplified through the Polymerase Chain Reaction (PCR) technique. In the second step, the parasite-Aps will be challenged against alpha-gal-1, mucin-1 and /or trans-sialidase and the new binding aptamers (parasite-molecule-Aps) will be amplified using PCR. The parasite-molecule-Aps will go through Flow cytometry, confocal microscopical analysis, and Single Photon Emission Computadorized Thomography (SPECT-CT) to confirm their T. cruzi binding capacity. All parasite-molecule-Aps will be finally tested on their capacity to inhibit T. cruzi infection in vitro. The development of this work may open new theranostic approaches applicable to combat Chagas disease.

KEYWORDS: Chagas disease; aptamers; PCR"

P47 - Expression and characterization of recombinant human blood coagulation Factor VII in human cell lines

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The human blood coagulation factor VII is a vitamin K dependent complex protein with different post-translational modifications (PTM's), such as N- and O-glycosylation and gamma-carboxilation. It's a therapeutic alternative for hemophilic patients who developed inhibitors against coagulation factors VIII (Hemophilia A) and IX (Hemophilia B).

The available recombinant FVII on the market is produced in mammalian cell lines, like Chinese Ovary Hamster (CHO) and Baby Hamster Kidney (BHK). Although they have similar structure compared to the protein found in humans, they can present different post-translational modifications (PTM's) that can cause immune response. The main objective of this work is to evaluate the production of factor VII of coagulation (rFVII) in four human cell lines: HKB-11, SK-Hep-1, Huh- 7 and HEK 293, all adapted to suspension serum-free conditions and already genetically modified for stable recombinant protein production (lentiviral transduction).

SK-Hep-1, HKB-11 and HEK 293 will be cultured in CDM4CHO (HyClone) medium and HUH-7 in CD 293 AGT (Gibco), all cells will be maintained in 5% CO2, 37°C environment and 150 rpm. The protein quantification and analysis will be done by ELISA and SDS-PAGE. The Biological activity of rFVII will be analyzed using the commercial kit Factor VII Human Chromogenic Activity Assay Kit (Abcam). The cell line that exhibits the best growth, rFVII production and biological activity will be cultivated in the 2L stirred tank bioreactor Celligen 310 (New Brunswick Scientific) under controlled cultivation conditions (pH and dissolved oxygen). After cultivation, the recombinant protein will be purified using an established protocol. Both the intact protein and the N-and O-glycosylated structures, as well as the content of monosaccharides will be characterized by mass spectrometry.

Before cultivation starts, a study of the cytotoxic effect of vitamin K in these cells lines was performed. Only Huh-7 were sensitive to the vitamin K.

Keywords: Recombinant coagulation factor VII, human cell lines, post-translational modifications, vitamin K

P48 - Construction and functional evaluation of a targeted drug delivery system for adenocarcinomas based on anti-tumor MUC1 recombinant antibody fragments

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Cancer is a severe disease associated with alterations in the molecular glycosylation patterns that lead to pathophysiologic consequences and to the discovery of cancer-biomarkers. Hypoglycosylated mucin 1 (MUC1 transmembrane glycoprotein 1) is an important tumor marker for several types of cancer. Among the most important challenges in cancer clinical research, we highlight the development of precise diagnostic and therapeutic strategies that allow the targeting of anti-tumor drugs direct to the cancer cell and not to healthy cells. Thus, the aim of this work is the development and the functional evaluation of an antitumor strategy based on the action of a targeted site-specific drug delivery system conjugated to anti-tumor marker (hypoglycosylated MUC1) antibody fragments. Preliminary data reports the selection, characterization and production of anti-MUC1 recombinant antibody fragments by Phage Display technique associated with next generation sequencing, in silico analysis and recombinant DNA technology. In this work, we improved the recombinant antibodies expression conditions for better yielding and analyzed their ability to bind three different cancer lineage cells with differential expression of MUC1. Next steps for the consolidation of this work proposal must be achieved through the immune characterization of recombinant antibodies by surface plasmon resonance and tissue microarray, as well as biological assays associated to radioisotopes for in vivo tumor localization. Finally, we intend to develop liposomes containing a given chemotherapeutic and conjugate the recombinant antibody on liposomes surface. This designed targeted drug delivery system will be evaluated in vitro and in vivo for its ability to achieve and promote tumor cells death. We understand that the obtainment of a drug delivery system, based or recombinant antibodies selected for hypoglycosylated MUC1, represents a novel potential biotechnological tool of clinical interest for the diagnosis and treatment of tumors without commitment of healthy cells.

Keywords: MUC1, cancer, recombinant antibody, drug delivery system

Financial Support: CAPES, FAPESP.

P49 - Development of real time RT-PCR for the differential diagnosis of chikungunya, dengue and Zika.

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Aims: Dengue fever is the most prevalent disease transmitted by the bite of female mosquitoes to humans; it's estimated that every year more than 390 million cases of Dengue virus (DENV) infections occurs, of which 96 million presents some clinical manifestation. Recently, Zika virus (ZIKV) and Chikungunya virus (CHIKV) were introduced in Brazil, causing several outbreaks. These viruses share the vector (Aedes aegypti) and induce symptoms similar to those of DENV. However, ZIKV is associated with microcephaly in newborns, while CHIKV with chronic arthralgia. Early diagnosis of infections caused by these viruses are important for a proper patient treatment and epidemics control. The aim of this work is to develop a real-time RT-PCR for the early diagnosis of infections with DENV, CHIKV and ZIKV.

Methods: Specific primers and probes were designed for CHIKV, ZIKV and for the four DENV serotypes from highly conserved genomic regions. A real-time RT-PCR was standardized for the detection of each virus, analyzing several reagent concentrations and cycling temperatures.

Results: The limit of detection of the real-time RT-PCR was 103 copies/mL for DENV-1, 102 copies/mL for DENV-2, 106 copies/mL for DENV-3 and DENV-4, 10-1 PFU/mL for CHIKV and 103 PFU/mL for ZIKV. The cross-reaction was analyzed by testing other flaviviruses and alphaviruses, showing the detection of only the viruses of interest.

Conclusions: The low limit of detection and the high specificity suggest a good perspective for the use of this protocol in differential diagnosis of dengue, chikungunya and Zika.

Keywords: virus; dengue; Zika; chikungunya; real time RT-PCR; diagnosis

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